

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**Applicant:** Joseph SCHLESSINGER, *et al.*  
**Title:** NOVEL RECEPTOR-TYPE PHOSPHOTYROSINE  
PHOSPHATASE-KAPPA ANTIBODIES  
**Prior Appl. Nos.:** 09/234,883; 08/087,244; and 08/049,384  
**Prior Filing Dates:** January 21, 1999; July 1, 1993; and April 21, 1993  
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**Art Unit:** Not Yet Assigned

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**PRELIMINARY AMENDMENT**

Commissioner for Patents  
Box PATENT APPLICATION  
Washington, D.C. 20231

Sir:

Prior to examination of the present application, Applicants respectfully request that the application be amended as follows:

**In the Title:**

Please amend the title as follows. A marked up version showing changes is attached:

NOVEL RECEPTOR-TYPE PHOSPHOTYROSINE PHOSPHATASE-KAPPA  
ANTIBODIES

**In the Specification:**

After the Application Title, please insert as follows. A marked up version showing changes is attached:

This is a Continuation Application of Application No. 09/234,883 filed January 21, 1999 which is, a Divisional Application of 08/087,244 filed July 1, 1993, now Patent No. 5,863,755, which is a continuation-in-part of Application No. 08/049,384, filed April 21, 1993, now abandoned.

Please amend the specification as follows:

On page 1, delete the first full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

The invention in the field of biochemistry and cell and molecular biology relates to novel receptor-type protein tyrosine phosphatase protein or glycoprotein, termed RPTP $\kappa$  (also known as RPTPase- $\kappa$ ), DNA coding therefore, methods for production and identification of the protein, methods for screening compounds capable of binding to and inhibiting or stimulating PTPase enzymatic activity, methods for inhibiting homophilic binding of RPTP $\kappa$ , and methods for identifying compounds which are capable of inhibiting homophilic RPTP $\kappa$  binding.

On page 8 and bridging page 9, delete the last full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

The invention is further directed to a nucleic acid molecule, preferably DNA, which may consist essentially of a nucleotide sequence encoding a mammalian RPTP $\kappa$  having the nucleotide sequence (SEQ ID NO:3) (FIG. 1(1) – 1(5)). Preferably, the nucleic acid molecule consists essentially of a nucleotide sequence encoding human RPTP $\kappa$  and having the nucleotide sequence (SEQ ID NO:4) or encodes a functional derivative thereof. The DNA molecule is preferably cDNA or genomic DNA. The invention is further directed to the DNA molecule in the form of an expression vehicle, as well as prokaryotic and eukaryotic hosts transformed or transfected with the DNA molecule.

On page 11, delete the second full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

FIG. 1A-1H shows the complete nucleotide sequence SEQ ID NO: 3 and amino acid sequence SEQ ID NO: 1 of murine RPTP $\kappa$ . The signal peptide, A5 homology region, transmembrane domain, and PTPase domains are designated by brackets.

On page 11, delete the fourth full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

FIG. 3 shows the predicted amino acid sequence (SEQ ID NO: 1) of the RPTP $\kappa$  precursor protein. The putative signal peptide and transmembrane (TM) segment are underlined. The two tandem phosphatase domains are boxed (PTP-1, PTP-2). The proteolytic cleavage site (RTKR 640-643) is printed in bold, and the Ig-like domain (Ig, 214-270) shown in bold italic characters. A5: homology to A5 surface protein (Takagi, S. *et al.*, 1991 Neuron 7:295-307); FN-III: fibronectin type III repeats. The Genbank accession number for the cDNA sequence is L10106.

On page 11 and bridging page 12, delete the last full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

FIG. 4 shows a proposed alignment of the four FN-III repeats of RPTP $\kappa$  (SEQ ID NO: 1) and domain 7 of human fibronectin (SEQ ID NO: 5) (Kornblihtt, A.R. *et al.*, 1985 EMBO J. 4:1755-1759). Residues most typically conserved in FN-III repeats are highlighted in bold. Residues identical in three or more out of the five aligned sequences are indicated with an asterisk. This region of the protein also contains clearly detectable homology to LAR, *Drosophila* PTPase 10D, and *Drosophila* neuroglian, all of which have been reported to contain FN-III repeats.

On page 12, delete the first full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

FIG. 5 shows an alignment of the N-terminal domains of RPTP $\kappa$  (SEQ ID NO: 1) and mRPTP $\mu$  (SEQ ID NO: 6) with the cell surface protein A5 (SEQ ID NO: 7) (Takagi *et al.*, *supra*). Numbers indicate the first residue of the respective proteins shown in the alignment. Residues marked as consensus are identical between A5 and RPTP $\kappa$ , or between A5 and mRPTP $\mu$ . Conservative substitutions are present but not shown. Residues in bold (C,W) define a possible Ig-like domain structure.

On page 13 and bridging page 14, delete the last full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

FIG. 9 shows RPTP $\kappa$  immunoreactive species in COS cells, and effect of Endo F treatment on SDS-PAGE mobility. Total lysates from mock or RPTP $\kappa$  transfected COS cells were treated or not with Endo F. The lysates were resolved by SDS-PAGE and immunoblotted with anti-N-terminal antibody 116 (left panel) or anti-cytoplasmic antibody 122 (right panel). The 95 kDa band in panel B also seen in mock-transfected cells is presumably due to fortuitous reactivity of antiserum 122 and not relevant to the analysis. No such protein species was detectable using an antiserum raised against the same antigen in a different rabbit.

FIG 10 shows results of a pulse-chase analysis of RPTP $\kappa$  processing. Mock-transfected with a wild type RPTP $\kappa$  expression vector (lanes 3 to 6) were metabolically labeled with [ $^{35}$ S]-methionine (200  $\mu$ Ci/ml) for 15 minutes ("pulse") and chased for the time-periods indicated. Immunoprecipitation was performed using antiserum 116. Arrows indicate the positions of the 210 kDa RPTP $\kappa$  precursor and the 110 kDa N-terminal cleavage product.

On page 14, delete the last full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

FIGS. 13A-13B is a series of micrographs showing the in situ hybridization analysis of RPTP $\kappa$  expression during development and in the adult CNS. 13A panel shows localization of RPTP $\kappa$  mRNA in the rat at embryonic day 18. CTX, cerebral cortex; MB, midbrain; SC, spinal cord; L, liver; K, kidney; I, intestine. 13B panel shows localization of RPTP $\kappa$  mRNA in a sagittal section of rat brain at postnatal day 6. CTX, cerebral cortex; CB, cerebellum; DG, dentate gyrus. In the cerebral cortex, particularly in the occipital region, the labeling is not uniform in all the cortical cell layers. In the hippocampal formation labeling is more intense in the dentate gyrus and in CA3. In the cerebellum, the most intense labeling is seen in the external granular cell layer.

On page 15, delete the second full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

FIGS. 15A-15E shows the nucleotide sequence of the human RPTP $\kappa$  (SEQ ID NO:4), designated MCP7, and its derived amino acid sequence (SEQ ID NO:2).

On page 15, delete the third full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

FIGS. 16A-16B. A comparison of the amino acid sequence of RPTP $\kappa$  SEQ ID NO:2 to the amino acid sequence of hRPTP $\mu$  SEQ ID NO:8. Lack of designation of an amino acid in hRPTP $\mu$  indicates identity to the MCP7 sequence. The putative signal peptide is overlined and dotted; the MAM domain is boxed with white background; the Ig-like domain is overlined with a shaded bar; the FN-III repeats are indicated with brackets above them; the RTKR cleavage site is underlined; the transmembrane domain is indicated with asterisks; and the PTPase domains are boxed. Both PTPase domains are shown with a shaded background.

On page 15 and bridging page 16, delete the last full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

FIGS. 19A-19B shows gel patterns indicating the transient expression of MCP7 mRNA in transfected cells. Cells of the 293 line were transfected with a MCP7 expression vector (or an empty vector as a control), metabolically labeled for 24 hours with [ $^{35}$ S] methionine and incubated with an anti-N-terminal antiserum 116. Cells were washed, lysed and protein-antibody complexes were removed by protein-A sepharose. Left panel shows a SDS-PAGE gel of immunoprecipitates. 19B panel shows Western blots of SDS-PAGE gels of lysates of cells transfected by MCP7-CMV (lane 1) or "empty" CMV (lane 2) and immunoblotted with the anti-N-terminal antiserum 116.

On page 16, delete the first full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

FIGS. 20A-20B shows Western blot patterns indicating co-expression of MCP7 with different RTKs. Semiconfluent 293 cells were transfected with expression plasmids encoding the indicated RTK together with either an equal amount of MCP7 expression vector or a control plasmid. After stimulation with the appropriate ligand: stem cell factor (SCF) for the p145.sup.c-kit RTK; epidermal growth factor for all other RTKs; insulin for I-R, cells were lysed, aliquots run on SDS-PAGE and transferred to nitrocellulose. Proteins were immunoblotted with anti-phosphotyrosine antibody 5E.2. Molecular mass markers are indicated.

On page 16, delete the second full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

FIGS. 21A-21B shows Northern blots indicating the relationship between MCP7 mRNA levels and the state of cell confluence in SK-BR-3 cells (left panel) and HT-29 cells (right panel) in culture. Poly(A)+ RNA (4 µg per lane) was prepared from cells obtained at different levels of confluence (lanes 1 and 4: 40%; lanes 2 and 5: 70%, lane 3 and 6: 100%) and was probed with a <sup>32</sup>P-labeled DNA probe corresponding to the extracellular domain of MCP7 (upper blots) and with a fragment coding for GAPDH (lower blots).

On page 19, delete the first full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

FIGS. 23A-23C. Aggregates consist solely of cells expressing the R-PTP $\kappa$  protein. Two different cell populations, one of which had been labeled with the fluorescent dye diI (J. Schlessinger *et al.* Science 195, 307, (1977)), were allowed to co-aggregate and the resulting aggregates inspected by visible and fluorescence microscopy. diI-fluorescence is white in the photographs.

FIG. 23A: a pool of R-PTP $\kappa$  expressing cells was allowed to aggregate in the presence of an equal number of diI-stained R-PTP $\kappa$  -negative cells.

FIG. 23B: R-PTP $\kappa$  expressing cells were stained with diI and allowed to aggregate in the presence of unstained RPTP $\kappa$ -negative cells.

FIG. 23C: mixture of stained and unstained R-PTP $\kappa$ -positive cells.

In each case, ten aggregates were randomly localized under visible light only. Subsequent inspection under U.V. light consistently showed the staining pattern exemplified in the photographs. dil dye (Molecular Probes, Inc.) was added to the growth medium at a concentration of 3.2  $\mu$ M during heat shock, and washed away prior to recovery and assay. 2 x 10<sup>6</sup> cells of each population were mixed and allowed to co-aggregate in a total volume of 1 ml.

On page 19 and bridging page 20, delete the last full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

FIGS. 24A-24D. Adhesion of R-PTP $\kappa$  transfected cells to a surface coated with recombinant purified R-PTP $\kappa$  extracellular domain protein. R-PTP $\kappa$  -negative, 1, or positive, 2, S2 cells, or R-PTP $\kappa$ -negative, 3, and positive, 4, L6 cells were incubated with a surface partially coated with the K2AP protein (circle), and the adherent cells fixed and stained. Amino acids 1-639 of the RPTP $\kappa$  proprotein were fused in-frame with human placental alkaline phosphatase in the vector pBacblue III (Invitrogen) by a series of appropriate cloning steps. Recombinant virus was generated and used to infect High-Five cells for production of the K2AP fusion protein using standard procedures. A secreted alkaline phosphatase (AP) control protein was generated in L6 myoblast cells by stable transfection with a modified version of the AP-TAG vector encoding a fusion protein of AP with a signal peptide. Both proteins were affinity purified by elution from an anti-alkaline phosphatase monoclonal antibody (Medix Biotech) column using 100 mM diethanolamine pH 11.5, or 50% ethylene glycol, dialyzed against PBS, and stored at 4° C. The K2AP and AP proteins were approximately 90% and 50% pure, resp. as determined by silver staining. To generate a mammalian cell line expressing the RPTP $\kappa$  protein, an MJ 30-based RPTP $\kappa$  expression vector was co-transfected with pSVneo into L6 cells, and individual clones surviving G418 selection screened for expression using immunoblotting. This procedure did not detect endogenous RPTP $\kappa$  protein in the parental L6 cells. The expressed protein underwent appropriate furin cleavage as described (Jiang, Y.-P. *et al.*, 1993, *Mol. Cell. Biol.* 13:2942).

On page 59, delete the second full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

The nucleotide sequence of murine RPTP $\kappa$  (SEQ ID NO:3) is shown in FIG. 1A-1H. The complete amino acid sequence of RPTP $\kappa$ . (SEQ ID NO:1) is shown in FIG. 1A-1H and in FIG. 3.

On page 60, delete the third full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

The first approximately 170 amino acids of RPTP $\kappa$  show similarity (26% overall identity) to a region in the *Xenopus* cell surface protein A5 with features of Ig-like domains (FIG. 5). The A5 protein (SEQ ID NO: 7) thought to function in recognition between input and target neurons in the visual system (Takagi, S. *et al.*, 1991 Neuron 7:295-307).

On page 62, delete the last full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

In order to assemble a full-length RPTP $\kappa$  cDNA from the various isolated fragments, a convenient fragment which included the N-terminus was generated from clone 35 by a PCR reaction using the N terminal primer

5'GAGCCGCGGCTCGAGTTAACCGCCATGGATGTGGCGGCCG3' (SEQ ID NO: 9)

and the C-terminal primer 5'GCTCACAGCTAGTTCAGCCC3' (SEQ ID NO: 10). This manipulation also removed all of the 5' untranslated sequences, while retaining an optimized Kozak consensus sequence for translation initiation (Kozak, M. 1983 *Microbiol. Rev.* 47:1-45).

On page 66, delete the first full paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

000001-000001



*In vitro* site-directed mutagenesis was performed using a commercially available kit from Clontech, using the manufacturer's instructions. An oligonucleotide having the sequence CTACACCCACATCTAACGAACCGTGAAGCAGGG (SEQ ID NO: 11) was used to modify the amino acid sequence RTKR in the cleavage site to the sequence LTNR. Mutagenesis was confirmed by direct DNA sequencing.

On page 68 and bridging page 69, delete the last paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

The level of expression of RPTP $\kappa$  mRNA was generally higher in the developing than in the adult central nervous system (CNS). At embryonic day 18 (E18) and at E20, the RPTP( $\kappa$ ) mRNA levels were highest in the cerebral cortex and hippocampal formation, followed by the cerebellum, brain stem and spinal cord. In the rest of the embryo, the highest levels were found in the liver, kidney and intestine (left panel, FIG. 13A). At postnatal day 6 (P6) and P8, expression was maximal in the cortex, olfactory bulb and hippocampal formation, especially in the dentate gyrus and CA3. In the cerebellum, the expression was highest in the granular cell layer, which in this stage of development still occupies the outermost cell layer of the cerebellum (right panel, FIG. 13B).

On page 77 and bridging page 78, delete the last paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

The nucleotide sequence of human RPTP $\kappa$ . (SEQ ID NO:4) is shown in FIG. 75A-E. The deduced amino acid sequence of MCP7 (SEQ ID NO:2) is also shown in FIG. 15A-E and displays the structural organization of a type II transmembrane PTPase (Fischer et al., 1991, Charbonneau, H. et al., Annu. Rev. Cell Biol. 8:463-493 (1992)). The N-terminal hydrophobic stretch of 20-26 amino acids is typical of signal peptides (von Heijne, G., J. Mol. Biol. 184:99-105 (1985)). A second region consisting of hydrophobic residues is found between positions 755 and 774 and is predicted to be a single .alpha. helical transmembrane domain. It is followed by a short region of mainly basic residues characteristic of a transfer stop sequence (Wickner, W. T. et al., Science 230:400-406 (1985)). The amino-terminal portion of the putative extracellular domain contains a sequence motif, a so called MAM

domain, spanning a region of about 170 residues. The MAM structural motif was recently established by comparison of several functionally diverse receptors (including RPTP.mu. and the A5 protein) and is thought to play a role in cell adhesion (Beckmann et al., supra). This motif is followed by one possible Ig-like domain (residues 207-277). The remaining extracellular portion contains conserved sequence motifs, indicating that it is composed of four FN-III related domains corresponding to the FN-III-like domains of LAR, PTP $\beta$  and RPTP $\mu$ . The extracellular domain contains 12 potential N-glycosylation sites, indicating that MCP7 is highly glycosylated. Interestingly, MCP7 contains the motif RXR/LR (residues 640-643) within the fourth FN-III domain. This motif has been described as the cleavage site for the subtilisin-like endoprotease, furin (Barr, P. J., Cell 66:1-3 (1991); Hosaka et al., supra).

On page 78 and bridging page 79, delete the last paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

The cytoplasmic part of MCP7 is composed of two PTPase domains containing the conserved amino acid sequences typical of all known PTPases (Saito, H. et al., Cell Growth Diff. 2:59-65 (1991)). A particularly intriguing feature is the region linking the transmembrane domain to the amino-terminal PTPase domain, which is nearly twice as large as that of most other receptor-like PTPases. A similar extended distance is shared only by the homologous PTPase, hRPTP $\mu$  (FIG. 16, 16A-B lower line). The overall homology between MCP7 and hRPTP.mu. is 77%, to which the N-terminal and C-terminal PTPase domains contribute 91% and 86%, respectively (FIG. 16A-B).

On page 84, delete the second paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

Two additional bands of 97 kDa and 116 kDa were immunoprecipitated (FIG. 19, 19A, lane 1); these bands were not detectable in cells transfected with a control vector. Such lower molecular weight products were thought to be cleavage products since the extracellular domain contains a common cleavage motif (RXR/LR; residues 640-643, FIG. 15A-15E). For processing by the endoprotease furin. These products are similar to the cleavage products

described above for murine RPTK. Furthermore, similar processing of the extracellular domain of LAR has been described (Streuli et al., *supra*).

On page 84 and bridging page 85, delete the last paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

The  $\alpha$  and  $\beta$  subunit are believed to form a stable complex, such that immunoprecipitation by an antibody specific for the extracellular domain would detect both subunits. To confirm that the 116 kDa band corresponded to the  $\alpha$  subunit cleavage product and not merely to a non-specifically cross-reacting species, lysates from MCP7 cDNA-transfected 293 cells were subjected to Western blots using antiserum 116 specific for an N-terminal epitope. With this approach, a band of about 116 kDa as well as an unprocessed precursor were found (FIG. 19B, right panel, lane 1), neither of which were detected in 293 cells at comparable levels transfected with a control vector (FIG. 19B, right panel, lane 2).

On page 86, delete the first paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

Co-expression of I-R, EGF-R, EP-R, EK-R, and SCF-R/c-kit with MCP7 resulted in a marked decrease in the ligand-induced receptor phosphotyrosine content when compared with control transfections in which MCP7 expression plasmid had been omitted (FIG. 20, 20A, lanes 1 and 9; 20B, lanes 1, 5, and 9). In contrast, HER1-2 appeared to be a poor substrate of MCP7, since only weak reduction of the ligand-induced phosphorylation state of this chimera was observed (FIG. 20A, lane 5). Interestingly, the intracellularly localized, incompletely processed precursor forms of I-R, EGF-R and EP-R (FIG. 20A, lanes 2, 4 and 10, 12; 20B, lanes 2, 4), as well as that of HER 1-2 (FIG. 20A, lanes 6, 8), were efficiently dephosphorylated), suggesting that MCP7 was present and active in the same intracellular compartments as the co-expressed RTKs before reaching the cell surface.

On page 87, delete the second paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

An equal number of SK-BR-3 cells was distributed onto either one, two, or four 15-cm dishes and incubated for two days under standard growth conditions. When harvested after two days, cells seeded at the various starting densities were found to be 100%, 70%, and 40% confluent, respectively. Poly(A)+RNA was prepared and Northern blot analysis was conducted as described supra, using a probe corresponding to the extracellular domain of MCP7. The results indicated that the level of MCP7 transcripts increased with increased cell density (FIG. 21A).

On page 87, delete the third paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

To determine whether this effect was unique to SK-BR-3 cells, an identical experiment was performed using the colon carcinoma-derived cell line HT 29. Expression of MCP7 mRNA was also found to be density-dependent with these cells (FIG. 19B).

On page 89 and bridging page 90, delete the last paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

In order to study whether RPTPk expression may mediate cell-cell aggregation, cells stably transfected with the RPTPk CDNA in either the sense orientation (sense cDNA) or the antisense orientation (antisense CDNA) were tested in an aggregation assay. uninduced and heat shock-induced cells were resuspended, subjected to rotary shaking to ensure mixing and to avoid adhesion to the vessel, and were then assayed for aggregate formation. The formation of a large number of aggregates consisting of more than 10 and up to approximately 100 cells was observed in heat-shocked sense cDNA-expressing cells only, whereas control cells (i.e., antisense cDNA transfected cells or non-heat shocked cells) remained essentially single cell suspensions (FIG. 22B-22C). Two methods of quantitation, counting of aggregates under the microscope, and determination of super-threshold particles with a Coulter-counter (FIG. 22C-22D) confirmed this conclusion. The fact that aggregation

was incomplete, with a large proportion of RPTP $\kappa$  transfected cells remaining as single cells throughout the assay period, is most likely due to the fact that the transfected cell population consisted of an uncloned pool of cells presumably differing in their levels of RPTP $\kappa$  expression. Notably, the conditions of the assay (i.e., medium, timescale, and speed of shaking) are similar to those used to demonstrate the adhesive properties of a number of well established adhesion molecules (H. Kramer, R. L. Cagan, S. L. Zipursky, *Nature* 352, 207 (1991); P. M. Snow, A. J. Bieber, C. Goodman, *Cell* 59, 313 (1989)). Therefore, in view of the difficulty of measuring binding affinities of many cell adhesion molecules which rely on cooperativity, it is likely that the strength of cell-cell-interaction conferred by expression of RPTP $\kappa$  is comparable to that of established, "classical", cell adhesion molecules.

On page 90 and bridging page 91, delete the last paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

The above experiments were performed with a full-length RPTP $\kappa$  cDNA, leaving unclear whether the phosphatase activity of the intracellular domain is required to confer adhesive properties. In several instances, an intact intracellular domain of cell adhesion molecules has in fact been shown to be required for certain aspects of cell-cell interaction (A. Nafaguchi and M. Takeichi, *EMBO J.* 7, 3679 (1988); S. H. Jaffe et al., *Proc. Natl. Acad. Sci. USA* 87, 3589 (1990), R. O. Hynes, *Cell* 69,111 (1992)). To test this issue, a cDNA encoding a mutant protein lacking most of the intracellular, catalytic, domain of RPTP $\kappa$  was constructed. FIG. 22E shows that such a truncation did not negatively interfere with cell aggregation as measured in this type of assay. The role of the furin cleavage site in the extracellular domain of RPTP $\kappa$  was also tested. Mutation of this site also left the adhesive behavior intact, suggesting that cleavage of the RPTP $\kappa$  proprotein (Y.-P. Jiang et al. *Mol. Cell. Biol.* 13, 2942 (1993)) is not required for induction of cell aggregation.

On page 91, delete the first paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

Cell adhesion molecules have been described which either do (e.g. cadherin family members and integrins), or do not (e.g. N-CAM, Ng-CAM) require the presence of Ca.sup.++

(G. M. Edelman, *Immun. Rev.* 100, 11 (1987); A. F. Williams and A. N. Barclay, *Annu. Rev. Immunol.* 6, 381 (1988); M. Grumet, *Curr. Opin. Neurobiol.* 1, 370 (1991); R. O. Hynes, *Cell* 69,111 (1992); B. Geiger and O. Ayalon, *Annu. Rev. Cell Biol.* 8 (1992)). The experiments presented in FIG. 22A-22E were performed in the presence of 10 mM Ca<sup>sup.++</sup> in the aggregating cell suspension. Performing a similar experiment in the absence of calcium ions and in the presence of 1 mM EGTA revealed no calcium requirement for RPTP $\kappa$  mediated cellular aggregation under the conditions of the assay.

On page 92 and bridging page 93, delete the last paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

It was next determined whether the extracellular domain of RPTP $\kappa$  was able to function by itself as a substrate for attachment of cells expressing the RPTP $\kappa$  protein independent of other factors to assist in the adhesion process. A baculovirus expression system was used to produce a soluble recombinant protein consisting of virtually the entire extracellular domain of the RPTP $\kappa$  protein, fused to placental alkaline phosphatase, which served as a tag for purification and detection (J. G. Flanagan and P. Leder, *Cell* 63, 185 (1990)). Fusion between the two protein moieties was designed to occur precisely before the furin proteolytic cleavage signal in the fourth fibronectin type III repeat in RPTP $\kappa$  (Y.-P. Jiang et al. *Mol. Cell. Biol.* 13, 2942 (1993)). The purified recombinant protein (K2AP) was used to coat bacteriological Petri dishes, and monitored for its ability to allow attachment of RPTP $\kappa$ -expressing S2 cells. Only induced, RPTP $\kappa$  expressing cells showed adhesive behavior to the K2AP coated surface (FIG. 24A-24D; Table II below).

On page 95, delete the first paragraph and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

No attachment occurred to control coated surfaces, which included alkaline phosphatase or the recombinant extracellular domain of human EGF-receptor (I. Lax et al., *J. Biol. Chem.* 266, 13828 (1991)), also purified by affinity chromatography from a baculovirus expression system. Whereas the above experiments were performed in the context of insect cells, the effect of RPTP $\kappa$  protein expression in mammalian cells in a similar cell-to-substrate

adhesion assay was also tested. In contrast to parental *Drosophila* S2 cells, rat L6 myoblast cells, the mammalian cell line used as a recipient for RPTP $\kappa$  overexpression, already shows a low level of spontaneous adhesion to a K2AP protein coated surface. However, stable overexpression of an RPTP $\kappa$  cDNA in these cells led to a significant (2.7 fold  $\pm$ 1.0; n=3) increase in adhesive capacity to a surface coated with the recombinant soluble extracellular domain of the RPTP $\kappa$  protein (FIG. 24A-24D).

#### In the Claims:

In accordance with 37 CFR §1.121, please substitute for original claims 1-12 for the following rewritten versions of the same claims, as amended. The changes are shown explicitly in the attached "Version with Markings to Show Changes Made."

1. (Amended) An isolated antibody which specifically binds to a mammalian protein or glycoprotein comprising the amino acid sequence of SEQ ID NO: 1.
2. (Amended) The antibody of claim 1, wherein the antibody is a monoclonal antibody.
3. (Amended) An isolated antibody which specifically binds to a mammalian receptor-type phosphotyrosine phosphatase  $\kappa$  protein or glycoprotein encoded by (a) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 3; or (b) a nucleic acid molecule comprising a nucleotide sequence which hybridizes to the complement of a nucleotide sequence that encodes the polypeptide of SEQ ID NO: 1 under hybridization conditions at least as stringent as the following: hybridization in 50% formamide, 5x SSC, 50mM NaH<sub>2</sub>PO<sub>4</sub>, pH 6.8, 0.5% SDS, 0.1 mg/ml sonicated salmon sperm DNA and 5x Denhart solution at 42°C overnight; washing with 2x SSC, 0.1% SDS at 45°C; and washing with 0.2x SSC, 0.1% SDS at 45°C.
4. (Amended) The antibody of claim 3, wherein the antibody is a monoclonal antibody.
5. (Amended) An isolated antibody which specifically binds to a protein or glycoprotein comprising the full length amino acid sequence of SEQ ID NO: 2, wherein said antibody binds to an epitope located within the amino acid sequence of SEQ ID NO:2.

6. (Amended) The antibody of claim 5, wherein the antibody is a monoclonal antibody.

7. (Amended) An isolated antibody which specifically binds to a mammalian receptor-type tyrosine phosphatase  $\kappa$  protein or glycoprotein encoded by (a) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 4; or (b) a nucleic acid molecule comprising a nucleotide sequence which hybridizes to the complement of a nucleotide sequence that encodes the polypeptide of SEQ ID NO:2 under hybridization conditions at least as stringent as the following: hybridization in 50% formamide, 5x SSC, 50mM  $\text{NaH}_2\text{PO}_4$ , pH 6.8, 0.5% SDS, 0.1 mg/ml sonicated salmon sperm DNA and 5x Denhardt's solution at 42°C overnight; washing with 2x SSC, 0.1% SDS at 45°C; and washing with 0.2x SSC, 0.1% SDS at 45°C.

8. (Amended) The antibody of claim 7, wherein the antibody is a monoclonal antibody.

9. (Amended) An isolated antibody which specifically binds to a mammalian protein comprising at least one of the following domains in SEQ ID NO: 1: the signal peptide domain, the MAM domain, the Ig-like domain, one of the four FN-type III like domains, the phosphatase I domain, the phosphatase II domain, the extracellular domain, the transmembrane domain or the intracellular domain, wherein said antibody binds to an epitope located within the amino acid sequence of SEQ ID NO:1.

10. (Amended) The antibody of claim 9, wherein the antibody is a monoclonal antibody.

11. (Amended) An isolated antibody which specifically binds to a mammalian protein comprising at least one of the following domains in SEQ ID NO:2: the signal peptide domain, the MAM domain, the Ig-like domain, one of the four FN-type III like domains, the phosphatase I domain, the phosphatase II domain, the extracellular domain, the transmembrane domain or the intracellular domain, wherein said antibody binds to an epitope located within the amino acid sequence of SEQ ID NO:2.

12. (Amended) The antibody of claim 11, wherein the antibody is a monoclonal antibody.



Please add the following new claims:

13. (New) The isolated antibody of claim 9 which binds to the signal peptide domain.
14. (New) The isolated antibody of claim 9 which binds to the MAM domain.
15. (New) The isolated antibody of claim 9 which binds to the Ig-like domain.
16. (New) The isolated antibody of claim 9 which binds to a FN-Type III domain.
17. (New) The isolated antibody of claim 9 which binds to the phosphatase I domain.
18. (New) The isolated antibody of claim 9 which binds to the phosphatase II domain.
19. (New) The isolated antibody of claim 9 which binds to the extracellular domain.
20. (New) The isolated antibody of claim 9 which binds to the transmembrane domain.
21. (New) The isolated antibody of claim 9 which binds to the intracellular domain.
22. (New) The isolated antibody of claim 11 which binds to the signal peptide domain.
23. (New) The isolated antibody of claim 11 which binds to the MAM domain.
24. (New) The isolated antibody of claim 11 which binds to the Ig-like domain.
25. (New) The isolated antibody of claim 11 which binds to a FN Type-III domain.
26. (New) The isolated antibody of claim 11 which binds to the phosphatase I domain.

27. (New) The isolated antibody of claim 11 which binds to the phosphatase II domain.
28. (New) The isolated antibody of claim 11 which binds to the extracellular domain.
29. (New) The isolated antibody of claim 11 which binds to the transmembrane domain.
30. (New) The isolated antibody of claim 11 which binds to the intracellular domain.
31. (New) An isolated antibody which specifically binds to a polypeptide comprising amino acids 60-76 of SEQ ID NO:1.
32. (New) An isolated antibody which specifically binds to a polypeptide amino acids 910-929 of SEQ ID NO:1.
33. (New) An isolated antibody which specifically binds to a mammalian protein or glycoprotein comprising the amino acid sequence of SEQ ID NO:1, wherein said antibody binds to the transmembrane domain.
34. (New) An isolated antibody which specifically binds to a mammalian protein or glycoprotein comprising the amino acid sequence of SEQ ID NO:2, wherein said antibody binds to the transmembrane domain.
35. (New) An isolated antibody which specifically binds to an extracellular domain of a mammalian protein or glycoprotein of SEQ ID NO:1.
36. (New) An isolated antibody which specifically binds to an extracellular domain of a mammalian protein or glycoprotein of SEQ ID NO:2.

**REMARKS**

Applicant respectfully requests that the foregoing amendments be made prior to examination of the present application.

Applicants believe that the present application is now in condition for allowance. Favorable consideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

By 

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Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Title:**

NOVEL RECEPTOR-TYPE PHOSPHOTYROSINE PHOSPHATASE-KAPPA  
ANTIBODIES

This is a Continuation Application of Application No. 09/234,883 filed January 21, 1999 which is, a Divisional Application of 08/087,244 filed July 1, 1993, now Patent No. 5,863,755.

Marked up replacement paragraphs:

**In the Specification:**

Please amend the specification as follows:

On page 1, the first full paragraph:

The invention in the field of biochemistry and cell and molecular biology relates to novel receptor-type protein tyrosine phosphatase protein or glycoprotein, termed RPTP $\kappa$  (also known as RPTPase- $\kappa$ ), DNA coding [therefor] therefore, methods for production and identification of the protein, methods for screening compounds capable of binding to and inhibiting or stimulating PTPase enzymatic activity, methods for inhibiting homophilic binding of RPTP $\kappa$ , and methods for identifying compounds which are capable of inhibiting homophilic RPTP $\kappa$  binding.

On page 8 and bridging page 9, the last full paragraph:

The invention is further directed to a nucleic acid molecule, preferably DNA, which may consist essentially of a nucleotide sequence encoding a mammalian RPTP $\kappa$  having the nucleotide sequence (SEQ ID NO:3) (FIG. 1(1) - 1(5)). Preferably, the nucleic acid molecule consists essentially of a nucleotide sequence encoding human RPTP $\kappa$  and having the nucleotide sequence (SEQ ID NO:4) or encodes a [functional] functional derivative thereof. The DNA molecule is preferably cDNA or genomic DNA. The invention is further directed to the DNA molecule in the form of an expression vehicle, as well as prokaryotic and eukaryotic hosts transformed or transfected with the DNA molecule.

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On page 11, the second full paragraph:

FIGS. 1(1) - 1(5)] 1A-1H shows the complete nucleotide sequence SEQ ID NO:3 and amino acid sequence SEQ ID NO:1 of murine RPTP $\kappa$ . The signal peptide, A5 homology region, transmembrane domain, and PTPase domains are designated by brackets.

On page 11, the fourth full paragraph:

FIG. 3 shows the predicted amino acid sequence (SEQ ID NO: 1) of the RPTP $\kappa$  precursor protein. The putative signal peptide and transmembrane (TM) segment are underlined. The two tandem phosphatase domains are boxed (PTP-1, PTP-2). The proteolytic cleavage site (RTKR 640-643) is printed in bold, and the Ig-like domain (Ig, 214-270) shown in bold italic characters. A5: homology to A5 surface protein (Takagi, S. *et al.*, 1991 Neuron 7:295-307); FN-III: fibronectin type III repeats. The Genbank accession number for the cDNA sequence is L10106.

On page 11 and bridging page 12, the last full paragraph:

FIG. 4 shows a proposed alignment of the four FN-III repeats of RPTP $\kappa$  (SEQ ID NO: 1) and domain 7 of human fibronectin (SEQ ID NO: 5) (Kornblihtt, A.R. *et al.*, 1985 EMBO J. 4:1755-1759). Residues most typically conserved in FN-III repeats are highlighted in bold. Residues identical in three or more out of the five aligned sequences are indicated with an asterisk. This region of the protein also contains clearly detectable homology to LAR, *Drosophila* PTPase 10D, and *Drosophila* neuroglian, all of which have been reported to contain FN-III repeats.

On page 12, the first full paragraph:

FIG. 5 shows an alignment of the N-terminal domains of RPTP $\kappa$  (SEQ ID NO: 1) and mRPTP $\mu$  (SEQ ID NO: 6) with the cell surface protein A5 (SEQ ID NO: 7) (Takagi *et al.*, *supra*). Numbers indicate the first residue of the respective proteins shown in the alignment. Residues marked as consensus are identical between A5 and RPTP $\kappa$ , or between A5 and mRPTP $\mu$ . Conservative substitutions are present but not shown. Residues in bold (C,W) define a possible Ig-like domain structure.

On pages 13 and bridging page 14, the last full paragraph:

FIG. 9 shows RPTP $\kappa$  immunoreactive species in COS cells, and effect of Endo F treatment on SDS-PAGE mobility. Total lysates from mock or RPTP $\kappa$  transfected COS cells were treated or not with Endo F. The lysates were resolved by SDS-PAGE and immunoblotted with anti-N-terminal antibody 116 (left panel) or anti-cytoplasmic antibody 122 (right panel). The 95 kDa band in panel B also seen in mock-transfected cells is presumably due to fortuitous reactivity of antiserum 122 and not relevant to the analysis. No such protein species was detectable using an antiserum raised against the same antigen in a different rabbit.

FIG 10 shows results of a pulse-chase analysis of RPTP $\kappa$  processing. Mock-transfected with a wild type RPTP $\kappa$  expression vector (lanes 3 to 6) were metabolically labeled with [ $^{35}$ S]-methionine (200  $\mu$ Ci/ml) for 15 minutes ("pulse") and chased for the time-periods indicated. Immunoprecipitation was performed using antiserum 116. Arrows indicate the positions of the 210 kDa RPTP $\kappa$  precursor and the 110 kDa N-terminal cleavage product.

On page 14, the last full paragraph:

FIGS. 13A–13B is a series of micrographs showing the in situ hybridization analysis of RPTP $\kappa$  expression during development and in the adult CNS. 13A [Left] panel shows [location] localization of RPTP $\kappa$  mRNA in the rat at embryonic day 18. CTX, cerebral cortex; MB, midbrain; SC, spinal cord; L, liver; K, kidney; I, intestine. 13B [Right] panel shows localization of RPTP $\kappa$  mRNA in a sagittal section of rat brain at postnatal day 6. CTX, cerebral cortex; CB, cerebellum; DG, dentate gyrus. In the cerebral cortex, particularly in the occipital region, the labeling is not uniform in all the cortical cell layers. In the hippocampal formation labeling is more intense in the dentate gyrus and in CA3. In the cerebellum, the most intense labeling is seen in the external granular cell layer.

On page 15, the second full paragraph:

FIGS. [15(1) – 15(3)] 15A-15E shows the nucleotide sequence of the human RPTP $\kappa$  (SEQ ID NO:4), designated MCP7, and its derived amino acid sequence (SEQ ID NO:2).

On page 15, the third full paragraph:

FIGS. 16A-16B. A comparison of the amino acid sequence of RPTP $\kappa$  SEQ ID NO:2 to the amino acid sequence of hRPTP $\mu$  SEQ ID NO:8. Lack of designation of an amino acid in hRPTP $\mu$  indicates identify to the MCP7 sequence. The putative signal peptide[,] [the cleavage site and the transmembrane region are underlined, the beginning of each FN-III repeat is indicated. Both PTPase domains are shown with a shaded background] is overlined and dotted; the MAM domain is boxed with white background; the Ig-like domain is overlined with a shaded bar; the FN-III repeats are indicated with brackets above them; the RTKR cleavage site is underlined; the transmembrane domain is indicated with asterisks; and the PTPase domains are boxed. Both PTPase domains are shown with a shaded background.

On pages 15 and bridging page 16, the last full paragraph:

FIG. 19A-19B shows gel patterns indicating the transient expression of MCP7 mRNA in transfected cells. Cells of the 293 line were transfected with a MCP7 expression vector (or an empty vector as a control), metabolically labeled for 24 hours with [<sup>35</sup>S] methionine and incubated with an anti-N-terminal antiserum 116. Cells were washed, lysed and protein-antibody complexes were removed by protein-A sepharose. Left panel shows a SDS-PAGE gel of immunoprecipitates. 19B [Right] panel shows Western blots of SDS-PAGE gels of lysates of cells transfected by MCP7-CMV (lane 1) or "empty" CMV (lane 2) and immunoblotted with the anti-N-terminal antiserum 116.

On page 16, the first full paragraph:

FIGS. 20A-20B shows Western blot patterns indicating co-expression of MCP7 with different RTKs. Semiconfluent 293 cells were transfected with expression plasmids encoding the indicated RTK together with either an equal amount of MCP7 expression vector or a control plasmid. After stimulation with the appropriate ligand: stem cell factor (SCF) for the p145<sub>sup.c-kit</sub> RTK; epidermal growth factor for all other RTKs; insulin for I-R, cells were lysed, aliquots run on SDS-PAGE and transferred to nitrocellulose. Proteins were immunoblotted with anti-phosphotyrosine antibody 5E.2. Molecular mass markers are indicated.

On page 16, the second full paragraph:

FIGS. 21A-21B shows Northern blots indicating the relationship between MCP7 mRNA levels and the state of cell confluence in SK-BR-3 cells (left panel) and HT-29 cells (right panel) in culture. Poly(A)<sup>+</sup> RNA (4 µg per lane) was prepared from cells obtained at different levels of confluence (lanes 1 and 4: 40%; lanes 2 and 5: 70%, lane 3 and 6: 100%) and was probed with a <sup>32</sup>P-labeled DNA probe corresponding to the extracellular domain of MCP7 (upper blots) and with a fragment coding for GAPDH (lower blots).

On page 19, the first full paragraph:

FIGS. 23A-23C. Aggregates consist solely of cells expressing the R-PTP $\kappa$  protein. Two different cell populations, one of which had been labeled with the fluorescent dye diI (J. Schlessinger *et al.* Science 195, 307 (1977), were allowed to co-aggregate and the resulting aggregates inspected by visible and fluorescence microscopy. diI-fluorescence is white in the photographs.

[Left] FIG. 23A: a pool of R-PTP $\kappa$  expressing cells was allowed to aggregate in the presence of an equal number of diI-stained R-PTP $\kappa$ -negative cells.

[Middle] FIG. 23B: R-PTP $\kappa$  expressing cells were stained with diI and allowed to aggregate in the presence of unstained RPTP $\kappa$ -negative cells.

[Right] FIG. 23C: mixture of stained and unstained R-PTP $\kappa$ -positive cells. In each case, ten aggregates were randomly localized under visible light only. Subsequent inspection under U.V. light consistently showed the staining pattern exemplified in the photographs. diI dye (Molecular Probes, Inc.) was added to the growth medium at a concentration of 3.2 µM during heat shock, and washed away prior to recovery and assay. 2 x 10<sup>6</sup> cells of each population were mixed and allowed to co-aggregate in a total volume of 1 ml.

On pages 19 and bridging page 20, the last full paragraph:

FIGS. 24A-24D. Adhesion of R-PTP $\kappa$  transfected cells to a surface coated with recombinant purified R-PTP $\kappa$  extracellular domain protein. R-PTP $\kappa$ -negative, 1, or positive, 2, S2 cells, or R-PTP $\kappa$ -negative, 3, and positive, 4, L6 cells were incubated with a surface partially coated with the K2AP protein (circle), and the adherent cells fixed and stained.



Amino acids 1-639 of the RPTP $\kappa$  proprotein were fused in-frame with human placental alkaline phosphatase in the vector pBacblue III (Invitrogen) by a series of appropriate cloning steps. Recombinant virus was generated and used to infect High-Five cells for production of the K2AP fusion protein using standard procedures. A secreted alkaline phosphatase (AP) control protein was generated in L6 myoblast cells by stable transfection with a modified version of the AP-TAG vector encoding a fusion protein of AP with a signal peptide. Both proteins were affinity purified by elution from an anti-alkaline phosphatase monoclonal antibody (Medix Biotech) column using 100 mM diethanolamine pH 11.5, or 50% ethylene glycol, dialyzed against PBS, and stored at 4° C. The K2AP and AP proteins were approximately 90% and 50% pure, resp. as determined by silver staining. To generate a mammalian cell line expressing the RPTP $\kappa$  protein, an MJ 30-based RPTP $\kappa$  expression vector was co-transfected with pSVneo into L6 cells, and individual clones surviving G418 selection screened for expression using immunoblotting. This procedure did not detect endogenous RPTP $\kappa$  protein in the parental L6 cells. The expressed protein underwent appropriate furin cleavage as described (Jiang, Y.-P. *et al.*, 1993, *Mol. Cell. Biol.* 13:2942).

On page 59, the second full paragraph:

The nucleotide sequence of murine RPTP $\kappa$  (SEQ ID NO:3) is shown in FIG. [1(1)] 1A-[1(5)] 1H. The complete amino acid sequence of RPTP $\kappa$ . (SEQ ID NO:1) is shown in FIG. [1(1) through] 1A-[1(5)] 1H and in FIG. 3.

On page 60, the third full paragraph:

The first approximately 170 amino acids of RPTP $\kappa$  show similarity (26% overall identity) to a region in the *Xenopus* cell surface protein A5 with features of Ig-like domains (FIG. 5). The A5 protein (SEQ ID NO: 7) is thought to function in recognition between input and target neurons in the visual system (Takagi, S. *et al.*, 1991 *Neuron* 7:295-307).

On page 62, the last full paragraph:

In order to assemble a full-length RPTP $\kappa$  cDNA from the various isolated fragments, a convenient fragment which included the N-terminus was generated from clone 35 by a PCR reaction using the N terminal primer  
5'GAGCCGCGGCTCGAGTTAACC GCCATGGATGTGGCGGCCG3' [(SEQ ID NO:5)]

(SEQ ID NO: 9) and the C-terminal primer 5'GCTCACAGCTAGTTCAGCCC3' [(SEQ ID NO:6)] (SEQ ID NO: 10). This manipulation also removed all of the 5' untranslated sequences, while retaining an optimized Kozak consensus sequence for translation initiation (Kozak, M. 1983 *Microbiol. Rev.* 47:1-45).

On page 66, the first full paragraph:

*In vitro* site-directed mutagenesis was performed using a commercially available kit from Clontech, using the manufacturer's instructions. An oligonucleotide having the sequence CTACCCACATCTAACGAACCGTGAAGCAGGG [(SEQ ID NO:7)] (SEQ ID NO: 11) was used to modify the amino acid sequence RTKR in the cleavage site to the sequence LTNR. Mutagenesis was confirmed by direct DNA sequencing.

On page 68 and bridging page 69, the last paragraph:

The level of expression of RPTP $\kappa$  mRNA was generally higher in the developing than in the adult central nervous system (CNS). At embryonic day 18 (E18) and at E20, the RPTP( $\kappa$ ) mRNA levels were highest in the cerebral cortex and hippocampal formation, followed by the cerebellum, brain stem and spinal cord. In the rest of the embryo, the highest levels were found in the liver, kidney and intestine (left panel, FIG. 13A). At postnatal day 6 (P6) and P8, expression was maximal in the cortex, olfactory bulb and hippocampal formation, especially in the dentate gyrus and CA3. In the cerebellum, the expression was highest in the granular cell layer, which in this stage of development still occupies the outermost cell layer of the cerebellum (right panel, FIG. 13B).

On page 77 and bridging page 78, the last paragraph:

The nucleotide sequence of human RPTP $\kappa$ . (SEQ ID NO:4) is shown in FIG. [15(1) – (3)] 75A-E. The deduced amino acid sequence of MCP7 (SEQ ID NO:2) is also shown in FIG. 15[(1) – (3)] A-E and displays the structural organization of a type II transmembrane PTPase (Fischer et al., 1991, Charbonneau, H. et al., *Annu. Rev. Cell Biol.* 8:463-493 (1992)). The N-terminal hydrophobic stretch of 20-26 amino acids is typical of signal peptides (von Heijne, G., *J. Mol. Biol.* 184:99-105 (1985)). A second region consisting of hydrophobic residues is found between positions 755 and 774 and is predicted to be a single [ $\alpha$ ].alpha. helical transmembrane domain. It is followed by a short region of mainly basic

residues characteristic of a transfer stop sequence (Wickner, W. T. et al., Science 230:400-406 (1985)). The amino-terminal portion of the putative extracellular domain contains a sequence motif, a so called MAM domain, spanning a region of about 170 residues. The MAM structural motif was recently established by comparison of several functionally diverse receptors (including RPTP[μ].mu. and the A5 protein) and is thought to play a role in cell adhesion (Beckmann et al., supra). This motif is followed by one possible Ig-like domain (residues 207-277). The remaining extracellular portion contains conserved sequence motifs, indicating that it is composed of four FN-III related domains corresponding to the FN-III-like domains of LAR, PTPβ and RPTPμ. The extracellular domain contains 12 potential N-glycosylation sites, indicating that MCP7 is highly glycosylated. Interestingly, MCP7 contains the motif RXR/LR (residues 640-643) within the fourth FN-III domain. This motif has been described as the cleavage site for the subtilisin-like endoprotease, furin (Barr, P. J., Cell 66:1-3 (1991); Hosaka et al., supra).

On page 78 and bridging page 79, the last full paragraph:

The cytoplasmic part of MCP7 is composed of two PTPase domains containing the conserved amino acid sequences typical of all known PTPases (Saito, H. et al., Cell Growth Diff. 2:59-65 (1991)). A particularly intriguing feature is the region linking the transmembrane domain to the amino-terminal PTPase domain, which is nearly twice as large as that of most other receptor-like PTPases. A similar extended distance is shared only by the homologous PTPase, hRPTPμ (FIG. 16, 16A-B lower line). The overall homology between MCP7 and hRPTP[μ].mu. is 77%, to which the N-terminal and C-terminal PTPase domains contribute 91% and 86%, respectively (FIG. 16A-B).

On page 84, the second full paragraph:

Two additional bands of 97 kDa and 116 kDa were immunoprecipitated (FIG. 19, 19A, [left panel] lane 1); these bands were not detectable in cells transfected with a control vector. Such lower molecular weight products were thought to be cleavage products since the extracellular domain contains a common cleavage motif (RXR/LR; residues 640-643, FIG. 15[(1)]A-15[(3)]E). [f]For processing by the endoprotease furin. These products are similar to the cleavage products described above for murine RPTPκ. Furthermore, similar processing of the extracellular domain of LAR has been described (Streuli et al., supra).

On page 84 and bridging page 85, the last full paragraph:

The  $\alpha$  and  $\beta$  subunit are believed to form a stable complex, such that immunoprecipitation by an antibody specific for the extracellular domain would detect both subunits. To confirm that the 116 kDa band corresponded to the  $\alpha$  subunit cleavage product and not merely to a non-specifically cross-reacting species, lysates from MCP7 cDNA-transfected 293 cells were subjected to Western blots using antiserum 116 specific for an N-terminal epitope. With this approach, a band of about 116 kDa as well as an unprocessed precursor were found (FIG. 19B, right panel, lane 1), neither of which were detected in 293 cells at comparable levels transfected with a control vector (FIG. 19B, right panel, lane 2).

On page 86, the first full paragraph:

Co-expression of I-R, EGF-R, EP-R, EK-R, and SCF-R/c-kit with MCP7 resulted in a marked decrease in the ligand-induced receptor phosphotyrosine content when compared with control transfections in which MCP7 expression plasmid had been omitted (FIG. 20, 20A, [upper panel] lanes 1 and 9; 20B, lanes 1, 5, and 9). In contrast, HER1-2 appeared to be a poor substrate of MCP7, since only weak reduction of the ligand-induced phosphorylation state of this chimera was observed (FIG. 20A, [upper panel] lane 5). Interestingly, the intracellularly localized, incompletely processed precursor forms of I-R, EGF-R and EP-R (FIG. 20A, [upper panel] lanes 2, 4 and 10, 12; 20B, [lower panel] lanes 2, 4), as well as that of HER 1-2 (FIG. 20A, [upper panel] lanes 6, 8), were efficiently dephosphorylated), suggesting that MCP7 was present and active in the same intracellular compartments as the co-expressed RTKs before reaching the cell surface.

On page 87, the second full paragraph:

An equal number of SK-BR-3 cells was distributed onto either one, two, or four 15-cm dishes and incubated for two days under standard growth conditions. When harvested after two days, cells seeded at the various starting densities were found to be 100%, 70%, and 40% confluent, respectively. Poly(A)+RNA was prepared and Northern blot analysis was conducted as described [supra] supra, [in Section 11.1.] using a probe corresponding to the extracellular domain of MCP7. The results indicated that the level of MCP7 transcripts increased with increased cell density (FIG. 21A [left panel]).

On page 87, the third full paragraph:

To determine whether this effect was unique to SK-BR-3 cells, an identical experiment was performed using the colon carcinoma-derived cell line HT 29. Expression of MCP7 mRNA was also found to be density-dependent with these cells (FIG. 19B [ , right panel]).

On page 89 and bridging page 90, the last full paragraph:

In order to study whether RPTP $\kappa$  expression may mediate cell-cell aggregation, cells stably transfected with the RPTP $\kappa$  CDNA in either the sense orientation (sense cDNA) or the antisense orientation (antisense CDNA) were tested in an aggregation assay. uninduced and heat shock-induced cells were resuspended, subjected to rotary shaking to ensure mixing and to avoid adhesion to the vessel, and were then assayed for aggregate formation. The formation of a large number of aggregates consisting of more than 10 and up to approximately 100 cells was observed in heat-shocked sense cDNA-expressing cells only, whereas control cells ([i.e.] i.e., antisense cDNA transfected cells or non-heat shocked cells) remained essentially single cell suspensions (FIG. 22B-22C). Two methods of quantitation, counting of aggregates under the microscope, and determination of super-threshold particles with a Coulter-counter (FIG. 22C-22D) confirmed this conclusion. The fact that aggregation was incomplete, with a large proportion of RPTP $\kappa$  transfected cells remaining as single cells throughout the assay period, is most likely due to the fact that the transfected cell population consisted of an uncloned pool of cells presumably differing in their levels of RPTP $\kappa$  expression. Notably, the conditions of the assay ([i.e.] i.e., medium, timescale, and speed of shaking) are similar to those used to demonstrate the adhesive properties of a number of well established adhesion molecules (H. Kramer, R. L. Cagan, S. L. Zipursky, Nature 352, 207 (1991); P. M. Snow, A. J. Bieber, C. Goodman, Cell 59, 313 (1989)). Therefore, in view of the difficulty of measuring binding affinities of many cell adhesion molecules which rely on cooperativity, it is likely that the strength of cell-cell-interaction conferred by expression of RPTP $\kappa$  is comparable to that of established, "classical", cell adhesion molecules.

On page 90 and bridging page 91, the last full paragraph:

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00007650-100701

The above experiments were performed with a full-length RPTP $\kappa$  cDNA, leaving unclear whether the phosphatase activity of the intracellular domain is required to confer adhesive properties. In several instances, an intact intracellular domain of cell adhesion molecules has in fact been shown to be required for certain aspects of cell-cell interaction (A. Nafaguchi and M. Takeichi, EMBO J. 7, 3679 (1988); S. H. Jaffe et al., Proc. Natl. Acad. Sci. USA 87, 3589 (1990), R. O. Hynes, Cell 69,111 (1992)). To test this issue, a cDNA encoding a mutant protein lacking most of the intracellular, catalytic, domain of RPTP $\kappa$  was constructed. FIG. 22[D]E shows that such a truncation did not negatively interfere with cell aggregation as measured in this type of assay. The role of the furin cleavage site in the extracellular domain of RPTP $\kappa$  was also tested. Mutation of this site also left the adhesive behavior intact, suggesting that cleavage of the RPTP $\kappa$  proprotein (Y.-P. Jiang et al. Mol. Cell. Biol. 13, 2942 (1993)) is not required for induction of cell aggregation.

On page 91, the first full paragraph:

Cell adhesion molecules have been described which either do (e.g. cadherin family members and integrins), or do not (e.g. N-CAM, Ng-CAM) require the presence of Ca<sub>sup</sub>++ (G. M. Edelman, Immun. Rev. 100, 11 (1987); A. F. Williams and A. N. Barclay, Annu. Rev. Immunol. 6, 381 (1988); M. Grumet, Curr. Opin. Neurobiol. 1, 370 (1991), R. O. Hynes, Cell 69,111 (1992), B. Geiger and O. Ayalon, Annu. Rev. Cell Biol. 8 (1992)). The experiments presented in FIG. 22A-22E were performed in the presence of 10 mM Ca<sub>sup</sub>++ in the aggregating cell suspension. Performing a similar experiment in the absence of calcium ions and in the presence of 1 mM EGTA revealed no calcium requirement for RPTP $\kappa$  mediated cellular aggregation under the conditions of the assay.

On page 92 and bridging page 93, the last full paragraph:

It was next determined whether the extracellular domain of RPTP $\kappa$  was able to function by itself as a substrate for attachment of cells expressing the RPTP $\kappa$  protein independent of other factors to assist in the adhesion process. A baculovirus expression system was used to produce a soluble recombinant protein consisting of virtually the entire extracellular domain of the RPTP $\kappa$  protein, fused to placental alkaline phosphatase, which served as a tag for purification and detection (J. G. Flanagan and P. Leder, Cell 63, 185 (1990)). Fusion between the two protein moieties was designed to occur precisely before the

furin proteolytic cleavage signal in the fourth fibronectin type III repeat in RPTP $\kappa$  (Y.-P. Jiang et al. Mol. Cell. Biol. 13, 2942 (1993)). The purified recombinant protein (K2AP) was used to coat bacteriological Petri dishes, and monitored for its ability to allow attachment of RPTP $\kappa$ -expressing S2 cells. Only induced, RPTP $\kappa$  expressing cells showed adhesive behavior to the K2AP coated surface (FIG. 24A-24D; Table II below).

On page 95, the first full paragraph:

No attachment occurred to control coated surfaces, which included alkaline phosphatase or the recombinant extracellular domain of human EGF-receptor (I. Lax et al., J. Biol. Chem. 266, 13828 (1991)), also purified by affinity chromatography from a baculovirus expression system. Whereas the above experiments were performed in the context of insect cells, the effect of RPTP $\kappa$  protein expression in mammalian cells in a similar cell-to-substrate adhesion assay was also tested. In contrast to parental Drosophila S2 cells, rat L6 myoblast cells, the mammalian cell line used as a recipient for RPTP $\kappa$  overexpression, already shows a low level of spontaneous adhesion to a K2AP protein coated surface. However, stable overexpression of an RPTP $\kappa$  cDNA in these cells led to a significant (2.7 fold  $\pm$  1.0; n=3) increase in adhesive capacity to a surface coated with the recombinant soluble extracellular domain of the RPTP $\kappa$  protein (FIG. 24A-24D).

#### In the Claims:

1. (Amended) An isolated antibody [specific for a protein] which specifically binds to a mammalian protein or glycoprotein comprising the amino acid sequence of SEQ ID NO: 1.
2. (Amended) The antibody of claim 1, wherein the antibody is a monoclonal antibody.
3. (Amended) An isolated antibody [specific for a naturally occurring] which specifically binds to a mammalian receptor-type phosphotyrosine phosphatase  $\kappa$  protein or glycoprotein encoded by (a) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 3; or (b) a nucleic acid molecule comprising a nucleotide sequence which hybridizes to the complement of a nucleotide sequence that encodes the polypeptide of

SEQ ID NO: 1 under hybridization conditions at least as stringent as the following: hybridization in 50% formamide, 5x SSC, 50mM NaH<sub>2</sub>PO<sub>4</sub>, pH 6.8, 0.5% SDS, 0.1 mg/ml sonicated salmon sperm DNA and 5x Denhart solution at 42°C overnight; washing with 2x SSC, 0.1% SDS at 45°C; and washing with 0.2x SSC, 0.1% SDS at 45°C.

4. (Amended) The antibody of claim 3, wherein the antibody is a monoclonal antibody.

5. (Amended) An isolated antibody [specific for] which specifically binds to a protein or glycoprotein comprising the full length amino acid sequence of SEQ ID NO: 2, wherein said antibody binds to an epitope located within the amino acid sequence of SEQ ID NO:2.

6. (Amended) The antibody of claim 5, wherein the antibody is a monoclonal antibody.

7. (Amended) An isolated antibody [specific for a] which specifically binds to a mammalian receptor-type tyrosine phosphatase  $\kappa$  protein or glycoprotein encoded by (a) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 4; or (b) a nucleic acid molecule comprising a nucleotide sequence which hybridizes to the complement of a nucleotide sequence that encodes the polypeptide of SEQ ID NO:2 under hybridization conditions at least as stringent as the following: hybridization in 50% formamide, 5x SSC, 50mM NaH<sub>2</sub>PO<sub>4</sub>, pH 6.8, 0.5% SDS, 0.1 mg/ml sonicated salmon sperm DNA and 5x Denhardt's solution at 42°C overnight; washing with 2x SSC, 0.1% SDS at 45°C; and washing with 0.2x SSC, 0.1% SDS at 45°C.

8. (Amended) The antibody of claim 7, wherein the antibody is a monoclonal antibody.

9. (Amended) An isolated antibody [specific for a] which specifically binds to a mammalian protein comprising at least one of the following domains in SEQ ID NO: 1: the signal peptide domain, the MAM domain, the Ig-like domain, one of the four FN-type III like domains, the phosphatase I domain, the phosphatase II domain, the extracellular domain, the transmembrane domain or the intracellular domain, wherein said antibody binds to an epitope located within the amino acid sequence of SEQ ID NO:1.



10. (Amended) The antibody of claim 9, wherein the antibody is a monoclonal antibody.

11. (Amended) An isolated antibody [specific for a] which specifically binds to a mammalian protein comprising at least one of the following domains in SEQ ID NO:2: the signal peptide domain, the MAM domain, the Ig-like domain, one of the four FN-type III like domains, the phosphatase I domain, the phosphatase II domain, the extracellular domain, the transmembrane domain or the intracellular domain, wherein said antibody binds to an epitope located within the amino acid sequence of SEQ ID NO:2.

12. (Amended) The antibody of claim 11, wherein the antibody is a monoclonal antibody.

Please add the following new claims:

13. (New) The isolated antibody of claim 9 which binds to the signal peptide domain.
14. (New) The isolated antibody of claim 9 which binds to the MAM domain.
15. (New) The isolated antibody of claim 9 which binds to the Ig-like domain.
16. (New) The isolated antibody of claim 9 which binds to a FN-Type III domain.
17. (New) The isolated antibody of claim 9 which binds to the phosphatase I domain.
18. (New) The isolated antibody of claim 9 which binds to the phosphatase II domain.
19. (New) The isolated antibody of claim 9 which binds to the extracellular domain.
20. (New) The isolated antibody of claim 9 which binds to the transmembrane domain.

21. (New) The isolated antibody of claim 9 which binds to the intracellular domain.
22. (New) The isolated antibody of claim 11 which binds to the signal peptide domain.
23. (New) The isolated antibody of claim 11 which binds to the MAM domain.
24. (New) The isolated antibody of claim 11 which binds to the Ig-like domain.
25. (New) The isolated antibody of claim 11 which binds to a FN Type-III domain.
26. (New) The isolated antibody of claim 11 which binds to the phosphatase I domain.
27. (New) The isolated antibody of claim 11 which binds to the phosphatase II domain.
28. (New) The isolated antibody of claim 11 which binds to the extracellular domain.
29. (New) The isolated antibody of claim 11 which binds to the transmembrane domain.
30. (New) The isolated antibody of claim 11 which binds to the intracellular domain.
31. (New) An isolated antibody which specifically binds to a polypeptide comprising amino acids 60-76 of SEQ ID NO:1.
32. (New) An isolated antibody which specifically binds to a polypeptide amino acids 910-929 of SEQ ID NO:1.
33. (New) An isolated antibody which specifically binds to a mammalian protein or glycoprotein comprising the amino acid sequence of SEQ ID NO:1, wherein said antibody binds to the transmembrane domain.

34. (New) An isolated antibody which specifically binds to a mammalian protein or glycoprotein comprising the amino acid sequence of SEQ ID NO:2, wherein said antibody binds to the transmembrane domain.

35. (New) An isolated antibody which specifically binds to an extracellular domain of a mammalian protein or glycoprotein of SEQ ID NO:1.

36. (New) An isolated antibody which specifically binds to an extracellular domain of a mammalian protein or glycoprotein of SEQ ID NO:2.

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